REFERENCES

Balber, A.E. (1972). Exp. Parasitol. *31*, 307–319.

Branche, C., Kohl, L., Toutirais, G., Buisson, J., Cosson, J., and Bastin, P. (2006). J. Cell Sci. *119*, 3443–3455.

Engstler, M., Thilo, L., Weise, F., Grunfelder, C.G., Schwarz, H., Boshart, M., and Overath, P. (2004). J. Cell Sci. *117*, 1105–1115.

Engstler, M., Pfohl, T., Herminghaus, S., Boshart, M., Wiegertjes, G., Heddergott, N., and Overath, P. (2007). Cell *131*, 505–515.

Lythgoe, K.A., Morrison, L.J., Read, A.F., and Barry, J.D. (2007). Proc. Natl. Acad. Sci. USA *104*, 8095–8100.

Matthews, K.R. (2005). J. Cell Sci. *118*, 283–290.

McCulloch, R. (2004). Trends Parasitol. *20*, 117–121.

McLintock, L.M., Turner, C.M., and Vickerman, K. (1993). Parasite Immunol. *15*, 475–480.

Natesan, S.K., Peacock, L., Matthews, K., Gibson, W., and Field, M.C. (2007). Eukaryot. Cell, in press. Published online September 28, 2007. 10.1128/EC.00213-07.

Webb, H., Carnall, N., Vanhamme, L., Rolin, S., Van Den Abbeele, J., Welburn, S., Pays, E., and Carrington, M. (1997). J. Cell Biol. *139*, 103–114.

HIV-1 Pathogenesis: The Complexities of the CCR5-CCL3L1 Complex

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The chemokine receptor CCR5 is the most important entry coreceptor for HIV-1 in vivo. Its chemokine ligands, including CCL3L1, efficiently inhibit infection by receptor blockade and downmodulation. However, in *Nature Immunology*, Dolan et al. (2007) present a large human-cohorts study that identifies entry-independent, CCR5-CCL3L1-dependent effects on cell-mediated immunity as a strong correlate of pathogenesis and point to additional influences of the CCR5-CCL3L1 axis on disease progression through undefined mechanisms.

CCR5 has been suborned as an entry coreceptor by primate immunodeficiency viruses (PIV), most notably HIV-1 (human immunodeficiency virus type 1). The viral gp120 glycoproteins bind CCR5 during the events that drive fusion of the virus and cell membranes, leading to infection (Hartley et al., 2005). CCR5 was probably the sole receptor for the primordial PIV from which present-day viruses evolved, but acquiring the ability to use CD4 (cluster of differentiation 4) as a primary receptor prior to CCR5 binding quite plausibly provided advantages to PIVs. By serving as a high-affinity binding site on target cells, CD4 speeds infection, and it also allows the highly conserved CCR5 site on gp120 to remain shielded from neutralizing antibodies until too little time and space remain for successful intervention. Hence, CCR5 clearly influences HIV-1 replication, and a series of genetic studies have shown it is a major determinant on the rate of progression of HIV-1-infected individuals to acquired immunodeficiency syndrome (AIDS) and death. CCR5 expression varies between individuals, principally because of sequence variations, within its promoter, that affect protein production; the less CCR5 expressed, the slower disease progresses. Moreover, a rare, protein-inactivating mutation, *CCR5-Δ32*, strongly protects against acquisition of HIV-1 infection by homozygous individuals (heterozygotes are not protected, but progress to disease less rapidly) (Kuhmann and Hartley, 2008).

CCR5 has chemokine ligands that reflect its natural role within the immune system: MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5). CCL3L1 and CCL4L1 are variant chemokines encoded by genes with varying copy numbers. These chemokines inhibit CCR5 use by HIV-1 through allosteric blockade and receptor downmodulation (Figure 1) (Hartley et al., 2005); the more chemokines present, the less HIV-1 replicates. Genetic studies show that the number of *CCL3L1* gene copies, and hence CCL3L1 expression levels, influences disease progression (Gonzalez et al., 2005).

But the CCR5-CCL3L1 system also has other effects on disease progression. In a major new study in a recent issue of *Nature Immunology*, over 2000 HIV-1-infected and control individuals were categorized by *CCR5* genotype and *CCL3L1* copy number (Dolan et al., 2007). The combination of a *CCR5* high-expression genotype with a low *CCL3L1* copy number was designated a high genetic risk; the converse (low CCR5, high CCL3L1) was designated a low risk. A high CCR5-expression genotype combined with a high CCL3L1 copy number, or low CCR5 with low *CCL3L1*, constituted moderate risk categories.

Viral load, baseline CD4 T cell count, and *CCR5-CCL3L1* genetic status were all good predictors of both time to AIDS and the slope of $CD4^+$ T cell decline (Dolan et al., 2007). However, *CCR5-CCL3L1* genetics predicted that viral load or baseline $CD4^+$ T cell counts only weakly. The relationship between cell-surface CCR5 expression and viral load is complex. In vitro, HIV-1 entry varies nonlinearly with CCR5 expression, and the crucial level of CCR5 is affected by CD4 availability; modeling suggests that three to six CCR5 molecules mediate entry of a single virion (Kuhmann et al., 2000). Furthermore, viral load is influenced by the number of target cells and their susceptibility to infection, and the more HIV-1 replicates, the more it deprives itself of targets for further expansion. Understanding this helps to explain the weak covariation between viral load and *CCR5-CCL3L1* status.

How might *CCR5-CCL3L1* genetics influence pathogenesis independently of HIV-1 entry efficiency and viral cytopathic effects on $CD4^+$ T cells? An obvious possibility is via the cell-mediated immune (CMI) system. The adaptive immune response to a virus, whether humoral or cell mediated, is also self-attenuating: The more replication, the stronger the immune response, but the stronger the immune response, the less replication. The influence of such complexities were minimized by measurement of a parameter, delayed type hypersensitivity (DTH) to unrelated antigens, which could reflect intrinsic CMI responsiveness. A strong inverse correlation was observed between *CCR5-CCL3L1* genetic risk and the strength of the DTH response; in the HIV-1-infected cohort, DTH correlated with time to AIDS and inversely with the $CD4^+$ T cell depletion rate (Dolan et al., 2007). Finally, after adjustment for the effect of genetic status on DTH, a residual viral load- and CMI-independent effect of *CCR5-CCL3L1* variation on disease progression was identified in the HIV-1-infected group (Dolan et al., 2007). The *CCR5-CCL3L1* axis therefore seems to modulate HIV-1 disease course in three partly independent ways, one involving the adaptive immune system.

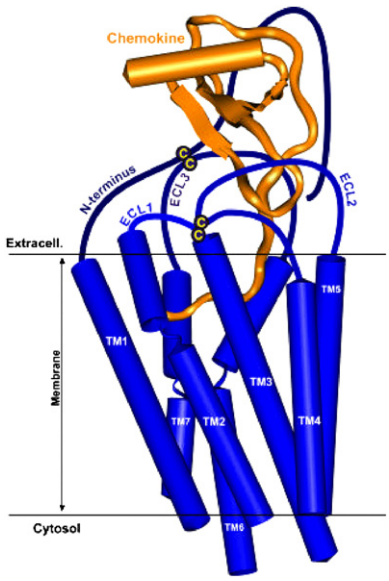

Figure 1. Hypothetical Molecular Model of Chemokine Bound to its Receptor

Chemokine receptors, e.g., CCR5, have seven α-helical transmembrane segments (blue cylinders); these are connected by intracellular and extracellular loops; the amino terminus is on the outside; the carboxy terminus on the inside (all in blue). The chemokine, e.g., CCL3L1, has three β strands and an α-helix (yellow). In the proposed interaction, the core of the chemokine contacts the extracellular loops, while its amino-terminal segment inserts itself into the bundle of helices. This binding has important consequences for HIV-1 infection and immune function. It signals via G proteins, blocks the binding of the HIV-1 envelope glycoprotein allosterically, and downmodulates the receptor from the cell surface. This image was reproduced with permission from Blanpain et al. (2003).

There has been a perception that CCR5 plays no role in the immune response to pathogens, on the basis of the observation that the rare humans (~1% of Caucasians) who are *CCR5-Δ32* homozygotes and so genetically lack CCR5 are healthy and live normal life spans. Moreover, CCR5 knockout mice are also "normal," and a significant minority of Red-cap mangabeys genetically lacks CCR5 without overt consequences. There are, however, both adverse and beneficial consequences of the absence of CCR5 under some circumstances. Thus, experimental infection of CCR5 knockout mice with various pathogens, such as *Cryptococcus neoformans*, can be lethal, whereas wild-type mice survive (Huffnagle et al., 1999), and *CCR5-Δ32* homozygous humans are at greater risk for lethal infection by West Nile virus (Glass et al., 2006). The loss of CCR5 arose in Northern Europe a few thousand years ago. The present-day geographic distribution and allele frequency reflect population migrations (Viking invasions) and a presumed beneficial effect of the absence of CCR5 on resistance to another disease, probably smallpox (although the Black Death has its proponents). If the absence of CCR5 can indeed sometimes help survival, this could be relevant to the conclusion that the less CCR5 expressed, the stronger the CMI response (Dolan et al., 2007). Exactly how *CCR5-CCL3L1* affects CMI responses to various pathogens, including HIV-1, is not known, but Dolan et al. (2007) note several possible mechanisms that warrant further investigation, including effects on T cell regeneration and the formation of the immunological synapse. Nevertheless, the beneficial effects of higher CCL3L1 levels, although complex, seem more straightforward than those of reduced CCR5 expression (Molon et al., 2005).

But what could the entry- and CMI-independent effects of *CCR5-CCL3L1* be? Here, we can only speculate, by pointing out factors that are probably irrelevant. For example, any effects of *CCR5-CCL3L1* on the size and susceptibility of the important T cell reservoir in gut-associated lymphoid tissue should be reflected in the resulting viral load, at least initially, as should the propensity of infected and bystander T cells to succumb to apoptotic or necrotic death, which would affect CMI as well. Likewise, modulation of adaptive or innate immunity that specifically dampens HIV-1 replication, without registering as DTH responsiveness, is plausible but should curb viral load. A switch from R5 to X4 virus is associated with increased disease progression but would not be favored by higher CCR5 levels. The parasite *Toxoplasma gondii* stimulates IL-12 (interleukin 12) secretion from dendritic cells through soluble factors that signal via CCR5 (Aliberti et al., 2000), but if this were a general mechanism, it would

presumably benefit host immunity and correlate directly, rather than inversely, with CCR5 expression.

Dolan et al. (2007) measure HIV-1 pathogenicity as a loss of $CD4^+$ T cells. Although many other immune system aberrations occur during HIV-1 infection, the elusive residual mechanism must therefore affect this particular central parameter. Because replication of a cytolytic virus is self-attenuating, fluctuations in viral load might occur as target cells in gut-associated lymphoid tissue are depleted early in infection. Such effects could cloud any simple correlation between viral load and the cellular susceptibility to HIV-1 infection or the rate of cell killing. Is the explanation for the residual effects of the *CCR5-CCL3L1* axis to be found in this complexity, or is there a novel mechanism? The biology underlying the new genetic observations clearly must be explored further. How *CCR5-CCL3L1* affects vaccine responses is also something to consider because there is considerable host-dependent variation in immune responses to HIV-1 vaccine immunogens.

An additional point is that antagonists of CCR5 function represent a new class of drugs for treating HIV-1 infection. Of such small-molecule CCR5 ligands, Maraviroc was recently licensed, Vicroviroc is in late stage trials, and others are in development (Kuhmann and Hartley, 2008). In a few individuals in these trials, the peripheral blood $CD4^+$ T cell count increases even when viral load is unchanged; this effect could be relevant to the findings of Dolan et al. (2007). To date, there have been few safety issues related to any adverse effect of CCR5 antagonists on immune function. Yet lingering concerns remain that interfering with CCR5 could be problematic under certain circumstances, such as infection with West Nile virus and related pathogens. As noted above, the complete absence of CCR5 is a European trait that is generally neutral or even beneficial to immune responses against pathogens that have circulated on that continent or that still do. This might not be true in a different protean environment, such as sub-Saharan Africa. Special care might therefore need to be taken if and when CCR5 antagonists are used in Africa. Although Dolan et al. (2007) conclude that the less CCR5 is expressed, the better the CMI response, this might not apply equally to every pathogen, particularly ones rare in or absent from the study cohorts.

This comprehensive new study should help improve our understanding of HIV-1 pathogenesis, susceptibility, and protective immunity, if only by revealing what we must learn if we are to design better immune-based interventions in the prevention and treatment arenas.

REFERENCES

Aliberti, J., Reis e Sousa, C., Schito, M., Hieny, S., Wells, T., Huffnagle, G.B., and Sher, A. (2000). Nat. Immunol. *1*, 83–87.

Blanpain, C., Doranz, B.J., Bondue, A., Govaerts, C., De Leener, A., Vassart, G., Doms, R.W., Proudfoot, A., and Parmentier, M. (2003). J. Biol. Chem. *278*, 5179–5187.

Dolan, M.J., Kulkarni, H., Camargo, J.F., He, W., Smith, A., Anaya, J.-M., Miura, T., Hecht, F.M., Mamtani, M., Pereyra, F., et al. (2007). Nat. Immunol. Published online October 21, 2007. 10.1038/ni1521.

Glass, W.G., McDermott, D.H., Lim, J.K., Lekhong, S., Yu, S.F., Frank, W.A., Pape, J., Cheshier, R.C., and Murphy, P.M. (2006). J. Exp. Med. *203*, 35–40.

Gonzalez, E., Kulkarni, H., Bolivar, H., Mangano, A., Sanchez, R., Catano, G., Nibbs, R.J., Freedman, B.I., Quinones, M.P., Bamshad, M.J., et al. (2005). Science *307*, 1434–1440.

Hartley, O., Klasse, P.J., Sattentau, Q.J., and Moore, J.P. (2005). AIDS Res. Hum. Retroviruses *21*, 171–189.

Huffnagle, G.B., McNeil, L.K., McDonald, R.A., Murphy, J.W., Toews, G.B., Maeda, N., and Kuziel, W.A. (1999). J. Immunol. *163*, 4642–4646.

Kuhmann, S.E., and Hartley, O. (2008). Annu. Rev. Pharmacol. Toxicol. *48*, 1611–1637.

Kuhmann, S.E., Platt, E.J., Kozak, S.L., and Kabat, D. (2000). J. Virol. *74*, 7005–7015.

Molon, B., Gri, G., Bettella, M., Gomez-Mouton, C., Lanzavecchia, A., Martinez, A.C., Manes, S., and Viola, A. (2005). Nat. Immunol. *6*, 465–471.